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Large-scale expression of recombinant sialyltransferases and comparison their kinetic properties with native enzymes.

Williams MA, Kitagawa H, Datta AK, Paulson JC, Jamieson JC.

Cytel Corporation, San Diego, California 92121, USA.

Values of Km were determined for three purified sialyltransferases and the corresponding recombinant enzymes. The enzymes were Gal beta 1-4GlcNAc alpha 2-6 sialyltransferase and Gal beta 1-3(4)GlcNAc alpha 2-3 sialyltransferase from rat liver; these enzymes are responsible for the attachment of sialic acid to N-linked oligosaccharide chains; and the beta 1-3GalNAc alpha 2-3 sialyltransferase from porcine submaxillary gland that is responsible for the attachment of sialic acid to O-linked glycoproteins and glycolipids. A procedure for the large scale expression of active sialyltransferases from recombinant baculovirus-infected insect cells is described. For the liver enzymes values of Km were determined using rat and human asialo alpha 1 acid glycoprotein and N-acetyllactosamin as variable substrates; lacto-N-tetraose was also used with the Gal beta 1-3(4)GlcNAc alpha 2-3 sialyltransferases. Antifreeze glycoprotein was used as the macromolecular acceptor for the porcine enzyme. Values for Km were also determined using CMP-NeuA as the variable substrate.

PMID: 8748151 [PubMed - indexed for MEDLINE]

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Molecular cloning and expression of chick Gal beta 1,3GalNAc alpha 2,3-sialyltransferase.

Kurosawa N, Hamamoto T, Inoue M, Tsuji S.

Frontier Research Program, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan.

A cDNA clone encoding chick Gal beta 1,3GalNAc alpha 2,3-sialyltransferase (ST3Gal I) was isolated from a chick embryo brain cDNA library. The cDNA sequence included an open reading frame coding for 342 amino acids, and the deduced amino acid sequence showed 64% identity with that of the mouse enzyme. Northern blot analysis of chick embryos revealed that the ST3Gal I gene was expressed in early embryonic stages. The identity of the enzyme was confirmed by construction of a recombinant sialyltransferase in which the N-terminal part including the cytoplasmic tail and signal anchor domain was replaced with an immunoglobulin signal peptide sequence. This enzyme expressed in COS-7 cells exhibited transferase activity similar to that of mouse ST3Gal I.

PMID: 7766661 [PubMed - indexed for MEDLINE]

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